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Productive Infection of CD34+-Cell-Derived Megakaryocytes by X4 and R5 HIV-1 Isolates

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The human immunodeficiency virus (HIV-1) causes various hematopoietic abnormalities, with thrombocytopenia (TP) occurring in 30% of infected individuals. In the present study, we aimed to determine whether HIV-1 in the bone marrow of TP patients can infect primary megakaryocytes *in vitro*, which may contribute to the development of thrombocytopenia. We amplified the V3 loop of HIV-1 envelope from the bone marrow of TP and non-TP patients and constructed recombinant viruses. We demonstrate that the bone marrow of TP and non-TP patients contained R5 strains, whereas X4 strains were present only in the bone marrow of TP patients. Furthermore, HIV-1 from the bone marrow of TP and non-TP patients infected megakaryocytes to similar levels, suggesting that the V3 loop of HIV-1 may not contain the viral determinants of HIV-associated TP. Chemokine receptor analysis determined that CD34+-cell-derived megakaryocytes express CD4, CXCR4, and CCR5 and are productively infected by both X4 and R5 HIV-1 isolates. Finally, we showed that CD34+-cell-derived megakaryocytes express the chemokine receptor CCR3.

INTRODUCTION

Human immunodeficiency virus (HIV-1) infection suppresses bone marrow hematopoiesis and causes various peripheral blood cytopenias (Harbol *et al.,* 1994; Fauci, 1996; Jenkins *et al.,* 1998; Koka *et al.,* 1998; Moses *et al.,* 1998). Thrombocytopenia (TP) is the most common form of cytopenia in HIV-1-infected patients and occurs in ~30% of cases (Ratner, 1989; Harbol *et al.,* 1994). HIV-associated TP (platelet count < 100,000 mm^3 in the absence of opportunistic infections, neoplasms, and antiretroviral drug therapy) is a multifactorial disorder involving immune destruction of platelets in the periphery and reduced thrombocytopoiesis in the bone marrow (Walsh *et al.,* 1984; Ratner, 1989; Ballem *et al.,* 1992; Zauli *et al.,* 1996). HIV-1 has been implicated in the reduction of bone marrow thrombocytopoiesis by several studies. HIV-1 transcripts have been detected in megakaryocytes of TP patients (Zucker-Franklin and Cao, 1989; Louache *et al.,* 1991) and ineffective thrombocytopoiesis has been reported in these patients (Zauli *et al.,* 1991; Ballem *et al.,* 1992; Cole *et al.,* 1998). In addition, megakaryocyte morphology has been reported to be abnormal in TP patients (Zucker-Franklin *et al.,* 1989) and zidovudine treatment has been shown to improve platelet counts in these patients (Oksenhendler *et al.,* 1989; Boyar and Beall, 1991). Most importantly, it has been suggested that HIV-1 infection of bone marrow megakaryocytes may contribute to the development of thrombocytopenia (Louache *et al.,* 1991; Monte *et al.,* 1992; Chelucci *et al.,* 1998). However, conflicting results have been reported on the ability of HIV-1 to infect primary megakaryocytes and their precursors (Louache *et al.,* 1992; Zauli *et al.,* 1992a; De Luca *et al.,* 1993; Zauli and Davis, 1993), questioning the hypothesis that HIV-1 infection of bone marrow megakaryocytes may be one of the contributing factors in the development of thrombocytopenia.

In this study we aimed to determine whether HIV-1 from the bone marrow of TP and non-TP patients could infect primary megakaryocytes *in vitro*. We analyzed HIV-1 from the bone marrow because it has been shown that the HIV-1 quasispecies varies in different cellular compartments (Donaldson *et al.,* 1994; Delwart *et al.,* 1998; Poss *et al.,* 1995; van't Wout *et al.,* 1998; Voulgaropoulou *et al.,* 1999). Recombinant HIV-1 strains were constructed using the hypervariable loop 3 (V3) of HIV-1 envelope from the bone marrow of TP and non-TP patients (Voulgaropoulou *et al.,* 1999). We showed that HIV-1 strains from the bone marrow of TP patients were more pathogenic *in vitro* than HIV-1 strains from the bone marrow of non-TP controls (Voulgaropoulou *et al.,* 1999). Specifically, four of six HIV-1 strains from the bone marrow of TP patients replicated in T-cell lines or primary macrophages, whereas HIV-1 strains from the bone marrow of non-TP patients did not replicate in T-cell lines and only one of five strains replicated in primary macrophages (Table 1) (Voulgaropoulou *et al.,* 1999). In this report we identified the chemokine receptor molecules utilized by bone marrow HIV-1 for entry into susceptible cells and the chemokine receptor molecules expressed on CD34+-cell-derived megakaryocytes. Finally, we showed that both X4 and R5 HIV-1 strains productively infected primary megakaryocytes *in vitro*.

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TABLE 1

Co-receptor Utilization of HIV-1 Molecular Clones

Molecular clone[a]	Tropism[b]	GHOST[c] CXCR4	CCR5	CCR3
A/BM9	T	+	+	+
B/BM1	PBMC	−	+	−
B/BM4	PBMC	−	+	−
B/BM8	T	+	+	−
C/BM6	M	−	+	−
C/BM11	M	−	+	−
D/BM12	PBMC	−	+	−
D/BM13	PBMC	−	+	−
J/BM2	−	−	+	−
J/BM11	PBMC	−	+	−
K/BM15	M	−	+	+
J/Bld47	T	−	+	−
K/Bld1	M	−	+	−
p120	T	+	+	+
p125	M	+	+	−

[a] HIV-1 molecular clones from TP patients include A/BM9, B/BM1, B/BM4, B/BM8, C/BM6, and C/BM11. BM designates bone marrow HIV-1 strains and Bld designates blood HIV-1 strains. p120 and p125 are T-cell and macrophage tropic laboratory adapted HIV-1 molecular clones (Voulgaropoulou *et al.,* 1999).

[b] HIV-1 molecular clones capable of replication in primary macrophages or T-cell lines are designated as M or T, respectively. HIV-1 molecular clones capable of replication in peripheral blood mononuclear cells only are designated as PBMC. Replication competent molecular clones incapable of replication in PBMC, macrophages, or T-cell lines are designated by dashes (Voulgaropoulou *et al.,* 1999).

[c] Dashes represent background levels of RT activity (3×10^3 to 5×10^3 cpm/ml) and + represents levels of RT activity above background.

RESULTS

Coreceptor utilization of bone marrow HIV-1 molecular clones

This study includes 6 HIV-1-infected patients. Patients A, B, and C were diagnosed with HIV-associated TP, Patient D was diagnosed with nocardia infection in the bone marrow, and Patients J and K were diagnosed with Hodgkin's disease (Voulgaropoulou *et al.,* 1999). Bone marrow aspirates and blood were obtained after informed consent, and DNA sequences encoding the V3 loop of HIV-1 envelope were amplified by nested polymerase chain reaction (Voulgaropoulou *et al.,* 1999). Eleven HIV-1 recombinant clones were constructed from the bone marrow of three TP (A, B, and C) and three non-TP patients (D, J, and K), and two recombinant clones were constructed from the blood of two patients (J and K) (Table 1) (Voulgaropoulou *et al.,* 1999). To identify the chemokine receptors utilized by the recombinant HIV-1 clones for entry into susceptible cells, GHOST cells expressing CXCR4, CCR5, and CCR3 chemokine receptors were infected with the molecular clones listed in Table 1. None of the molecular clones was able to replicate in GHOST parental cells, except p120, which replicated to low levels due to the presence of endogenous CXCR4 on the surface of these cells (data not shown). All bone marrow and blood isolates were able to replicate in GHOST–CCR5 cells (R5) regardless of their ability to replicate in primary macrophages (Table 1). The T-cell tropic isolates A/BM9 and B/BM8 were able to replicate in GHOST–CXCR4 cells (X4), whereas the T-cell tropic isolate J/Bld47 did not replicate in these cells, suggesting that J/Bld47 was unable to utilize CXCR4 for entry into GHOST cells (Table 1). Finally, one TP (A/BM9) and one non-TP (K/BM15) isolate replicated in GHOST–CCR3 cells (Table 1). The reference T-cell tropic molecular clone p120 (Voulgaropoulou *et al.,* 1999) was able to replicate in GHOST–CXCR4 cells very efficiently but less efficiently in GHOST–CCR5 and -CCR3 cells, suggesting that p120 can utilize mainly CXCR4 but also CCR5 and CCR3 to enter susceptible cells (Table 1). The reference macrophage tropic molecular clone p125 (Voulgaropoulou *et al.,* 1999) was able to replicate in GHOST–CCR5 cells very efficiently but less efficiently in GHOST–CXCR4 cells, suggesting that p125 can utilize mainly CCR5 but also CXCR4 for entry into susceptible cells (Table 1). None of the molecular clones was able to replicate in GHOST cells expressing CCR1, CCR2b, CCR8, BOB, BONZO, and V28 chemokine receptors except p120, which was able to replicate in GHOST–CCR1 and -CCR2b cells (data not shown). Therefore the bone marrow of TP patients is characterized by the presence of both X4 and R5 HIV-1 strains whereas, only R5 HIV-1 strains were present in the bone marrow of non-TP patients.

Chemokine receptor profile of CD34+-cell-derived megakaryocytes

CD34+ hematopoietic progenitor cells were grown in serum-free media supplemented with 50 μg/ml thrombopoietin (Tpo) for 7 days. Under these conditions, CD34+ hematopoietic progenitor cells have been shown to differentiate into CD41+ megakaryocytes (Zucker-Franklin and Kaushansky, 1996; Birkmann *et al.,* 1997; Zauli *et al.,* 1997; Schipper *et al.,* 1998) (Fig. 1). Furthermore, CD34+-cell-derived megakaryocytes have been shown to be positive for CD4, the receptor for HIV-1 (Gewirtz *et al.,* 1992; Kouri *et al.,* 1993; Zauli *et al.,* 1995; Basch *et al.,* 1996; Lee *et al.,* 1999) (Fig. 1). To determine the chemokine receptor profile of primary megakaryocytes, CD34+-cell-derived megakaryocytes were labeled by immunofluorescence using CXCR4-, CCR5-, and CCR3-specific monoclonal antibodies. Confocal microscopy determined that the chemokine receptors CXCR4, CCR5, and CCR3 were present in 100% of CD34+-cell-derived megakaryocytes (Fig. 1). Therefore primary megakaryocytes express CD4, CXCR4, CCR5, and

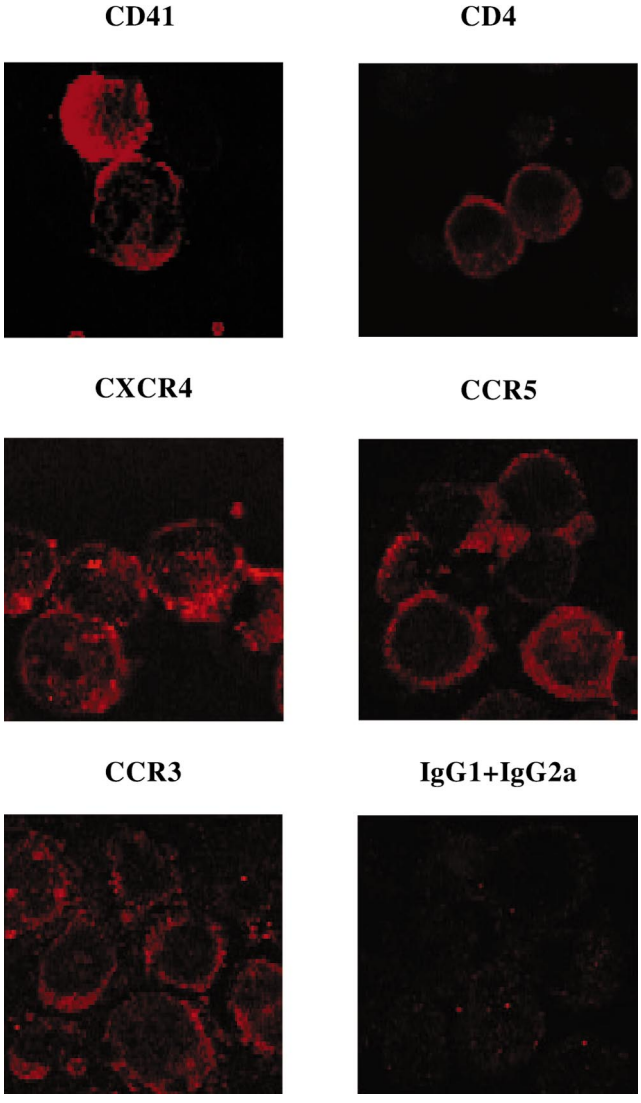

FIG. 1. Antibody staining of CD34+ hematopoietic progenitor cells grown in Tpo media for 7 days. The mouse monoclonal antibodies used were CD41, CD4, CXCR4, CCR5, and CCR3 (1–2 μg of antibody per 10^6 cells). IgG1 and IgG2a (1–2 μg of antibody per 10^6 cells) were used as isotype-matched negative control antibodies. Cells were stained with anti-mouse IgG conjugated to PE (1:100 dilution).

CCR3 and may be susceptible to infection by both X4 and R5 HIV-1 strains.

Infection of CD34+-cell-derived megakaryocytes by HIV-1

To determine whether CD34+-cell-derived megakaryocytes were susceptible to infection by X4 and/or R5 HIV-1 strains, recombinant virus from the bone marrow of HIV-1-infected patients was used for *in vitro* infections. Infection of megakaryocytes by HIV-1 was detected by double-labeled immunofluorescence using CD41 and HIV-1 specific antibodies and cell staining was analyzed by confocal microscopy. Figure 2 shows that CD41+ megakaryocytes were positive for staining by HIV-1 antisera following infection by both X4 and R5 HIV-1 strains. Furthermore, HIV-1 strains from the bone marrow of TP patients infected megakaryocytes to similar levels as HIV-1 strains from the bone marrow and blood of non-TP patients and the laboratory-adapted molecular clones p120 and p125. Viral release from HIV-1-infected megakaryocytes was measured to be ~190 pg of p24 per milliliter of culture supernatant, for all molecular clones studied (data not shown). Furthermore, the p24 value was similar for all time points tested during the 13-day infection (Days 3, 6, 9, and 13) for all molecular clones, suggesting that HIV-1 was constantly released from the infected megakaryocytes into the supernatant (data not shown). These results demonstrate that CD41+ megakaryocytes were productively infected by both X4 and R5 HIV-1 isolates *in vitro*.

DISCUSSION

The primary determinant of HIV-1 pathogenesis is the entry of HIV-1 into susceptible cells. HIV-1 entry is facilitated through the interaction of the HIV-1 envelope glycoprotein on the surface of the virion with the HIV-1 receptor molecules on the surface of target cells (Cairns and D'Souza, 1998; Lee *et al.,* 1998). The HIV-1 cellular receptors have been identified as the differentiation marker CD4 (Dalgleish *et al.,* 1984; Klatzmann *et al.,* 1984; Maddon *et al.,* 1986) and the chemokine receptors CXCR4 and CCR5 (Deng *et al.,* 1996; Dragic *et al.,* 1996; Feng *et al.,* 1996). Other chemokine receptor molecules have also been shown to function as HIV-1 coreceptors *in vitro* (Choe *et al.,* 1996; Doranz *et al.,* 1996; Deng *et al.,* 1997; Frade *et al.,* 1997; He *et al.,* 1997; Liao *et al.,* 1997; Loetscher *et al.,* 1997; McKnight *et al.,* 1997; Rucker *et al.,* 1997; Horuk *et al.,* 1998; Jinno *et al.,* 1998) but the biological significance of these molecules *in vivo* has not been established. The available evidence suggests that CXCR4 and CCR5 are the relevant chemokine receptors *in vivo* (Huang and Carmichael, 1996; Samson *et al.,* 1996; Zhang *et al.,* 1996; Smith *et al.,* 1997; Stewart *et al.,* 1997; Michael *et al.,* 1998; Mummidi *et al.,* 1998; Zhang and Moore, 1999) and are utilized by most or all HIV-1 isolates (Rucker *et al.,* 1997; Xiao *et al.,* 1998). In addition to CXCR4 and CCR5, the chemokine receptor CCR3 has been suggested to play a role in HIV-1 neuro tropism (He *et al.,* 1997; Shieh *et al.,* 1998; Zhang *et al.,* 1998). Generally, CXCR4 mediates entry of T-cell tropic, syncytia-inducing (SI) isolates into CD4+ cells, whereas CCR5 mediates entry of macrophage tropic, non-syncytia, inducing (NSI) isolates into these cells (Alkhatib *et al.,* 1996; Deng *et al.,* 1996; Dragic *et al.,* 1996; Feng *et al.,* 1996; Speck *et al.,* 1997).

In the present study, we aimed to determine whether HIV-1 in the bone marrow of TP patients can infect primary megakaryocytes *in vitro*, which may contribute to the development of thrombocytopenia. We studied two

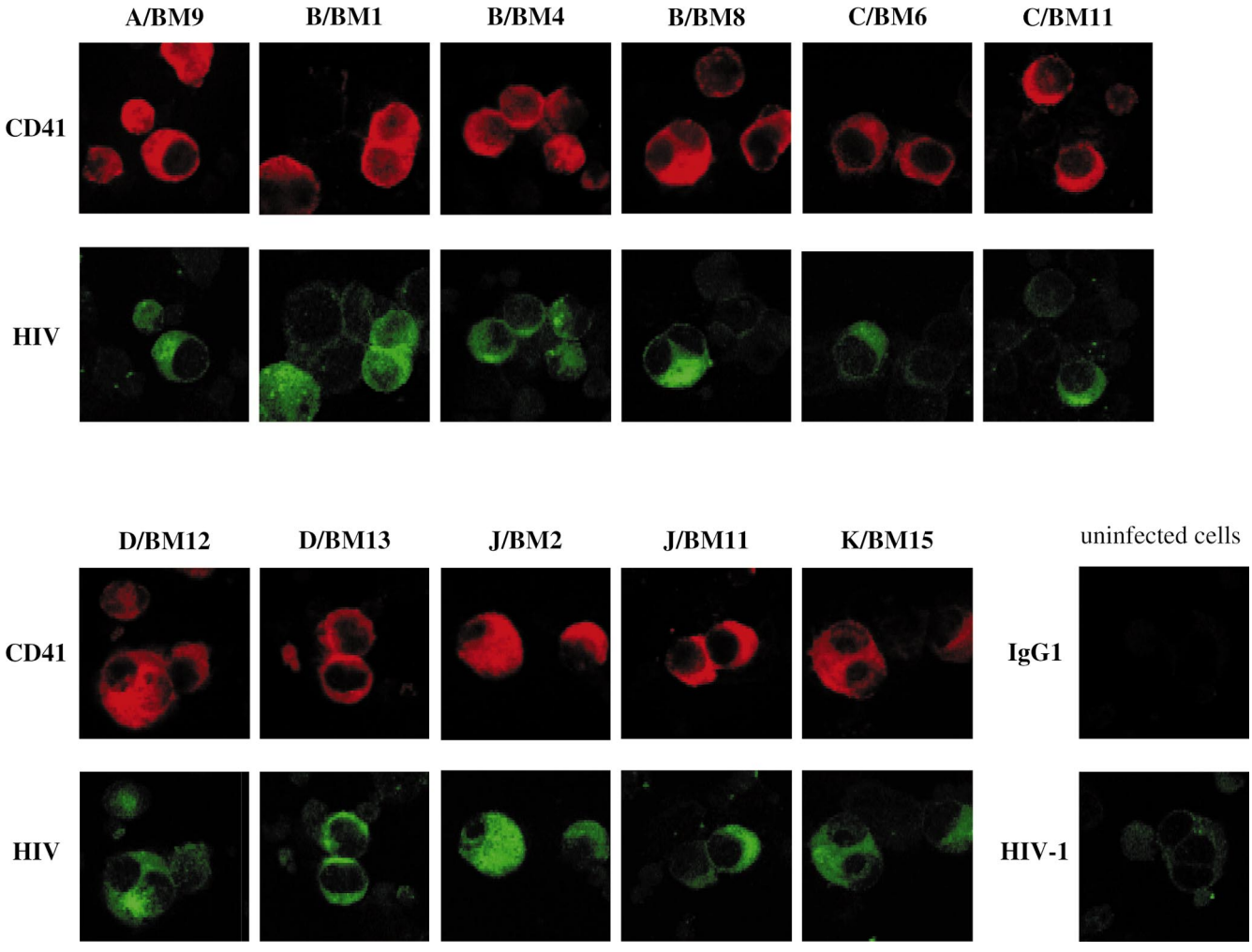

FIG. 2. HIV-1 infection of CD34+-cell-derived megakaryocytes. HIV-1-infected megakaryocytes were detected by two-color immunofluorescence using CD41 monoclonal antibody (1–2 μg/10^6 cells) and pooled human sera from HIV-1-infected patients (1:100 dilution). Uninfected cells stained with IgG1 isotype-matched antibody and human sera from HIV-1-infected patients served as negative control. The mouse monoclonal antibody CD41 was labeled with anti-mouse IgG conjugated to PE and HIV-1 antibodies were labeled with anti-human IgG conjugated to FITC (1:100 dilution).

groups of patients, patients with TP (A, B, and C) and non-TP patients with hematopoietic dysfunction caused by the invasion of bone marrow with neoplasms or opportunistic pathogens (D, J, and K). We constructed recombinant viruses using the V3 loop of HIV-1 envelope from the bone marrow and blood of these patients (Voulgaropoulou *et al.,* 1999) and determined the chemokine receptor molecules utilized by the bone marrow and blood HIV-1 strains and the chemokine receptor molecules expressed on megakaryocytes. We showed that all bone marrow and blood HIV-1 isolates utilize CCR5 for entry into susceptible cells, irrespective of their ability to replicate in primary macrophages. The use of CCR5 by most HIV-1 primary isolates has been reported by others (Simmons *et al.,* 1996; Clapham and Weiss, 1997; Cairns and D'Souza, 1998) as well as the inability of several R5 HIV-1 strains to infect macrophages (Cheng-Mayer *et al.,* 1997; Dittmar *et al.,* 1997; Smyth *et al.,* 1998; Hung *et al.,* 1999). The T-cell tropic HIV-1 strains, characteristic of TP patients, utilized CXCR4 for entry into susceptible cells, whereas one TP and one non-TP HIV-1 strain utilized CCR3. One notable exception was the T-cell tropic blood isolate J/Bld47, which was able to replicate in MT-2 cells but not in Jurkat and Hut78 T-cell lines (Voulgaropoulou *et al.,* 1999) and not in GHOST–CXCR4 cells (Table 1). The ability of X4 HIV-1 isolates to infect some T-cell lines but not others has been reported by others (Cheng-Mayer *et al.,* 1988; Connor *et al.,* 1993; Dumitrescu *et al.,* 1994). Finally, we did not observe a correlation between CCR3 utilization and thrombocytopenia because CCR3 utilizing HIV-1 strains were isolated from both TP and non-TP patients. Therefore chemokine receptor analysis revealed that X4 HIV-1 strains were present only in the bone marrow of TP patients, whereas R5 HIV-1 strains were present in the bone marrow of both TP and non-TP patients.

To determine the susceptibility of megakaryocytes to HIV-1 infection, we analyzed the chemokine receptor

profile of megakaryocytes using confocal microscopy. Megakaryocytes were obtained from CD34+ hematopoietic progenitor cells grown in serum-free medium supplemented with Tpo. Under these conditions, CD34+ progenitor cells differentiate into CD41+ megakaryocytes after several days in culture (Zucker-Franklin and Kaushansky, 1996; Birkmann *et al.,* 1997; Zauli *et al.,* 1997; Schipper *et al.,* 1998). Most importantly, these CD34+-cell-derived megakaryocytes have been reported to be morphologically identical to bone marrow megakaryocytes and to produce platelets *in vitro* that are indistinguishable from normal platelets (Choi *et al.,* 1995a,b; Cramer *et al.,* 1997). We showed, in agreement with published reports, that CD41+ megakaryocytes express surface CD4, CXCR4, and CCR5 (Gewirtz *et al.,* 1992; Kouri *et al.,* 1993; Zauli *et al.,* 1995; Basch *et al.,* 1996; Wang *et al.,* 1998; Kowalska *et al.,* 1999; Lee *et al.,* 1999; Riviere *et al.,* 1999) and may be susceptible to infection by both X4 and R5 HIV-1 strains. In addition, we report for the first time, the presence of the CCR3 chemokine receptor on CD34+-cell-derived megakaryocytes. Our results contradict the findings of Chelucci *et al.,* who did not detect CCR3 mRNA on cells of the megakaryocytic lineage (Chelucci *et al.,* 1999). Furthermore, Chelucci *et al.* (1999) did not detect CCR5 on the surface of megakaryocytes, an observation that contradicts our results and the results of others (Lee *et al.,* 1999).

To determine whether primary megakaryocytes were susceptible to infection by HIV-1, we exposed CD34+-cell-derived megakaryocytes to HIV-1 from the bone marrow of TP and non-TP patients. We showed that CD41+ megakaryocytes were productively infected by both X4 and R5 HIV-1 strains. Our results contradict previous reports on the susceptibility of megakaryocytes to T-cell tropic but not macrophage tropic HIV-1 (Chelucci *et al.,* 1998, 1999; Lee *et al.,* 1999). Furthermore, HIV-1 strains from the bone marrow of TP and non-TP patients infected megakaryocytes to similar levels. Because we did not observe a difference in the ability of HIV-1 from TP or non-TP patients to infect megakaryocytes, we believe that the viral determinants of HIV-associated TP may lie outside the V3 loop of HIV-1 envelope. Alternatively, HIV-1 may cause TP indirectly as has been suggested by others (Molina *et al.,* 1990; Louache *et al.,* 1992; Zauli *et al.,* 1992b,c).

In conclusion, we showed that the bone marrow of TP patients is characterized by the presence of both X4 and R5 HIV-1 strains, whereas only R5 HIV-1 strains were present in the bone marrow of non-TP patients. HIV-1 from the bone marrow of TP and non-TP patients infected megakaryocytes to similar levels, suggesting that the viral determinants HIV-associated TP may lie outside the V3 loop of the HIV-1 envelope. Furthermore, CD34+-cell-derived megakaryocytes express CD4, CXCR4, and CCR5 and are productively infected by both X4 and R5 HIV-1 strains. Finally, we showed that CD34+-cell-derived megakaryocytes express the chemokine receptor CCR3.

MATERIALS AND METHODS

Infection of GHOST cells with recombinant HIV-1

The GHOST HIV indicator cells [GHOST (3) parental, CXCR4, Hi-CCR5, and CCR3 (AIDS Research and Reference Reagent Program)] were used to determine the coreceptor molecules utilized by the HIV-1 recombinant clones for entry into susceptible cells. Briefly, 5×10^4 cells were infected with ~400 ng of HIV-1 $p24^{gag}$ overnight in the presence of Polybrene (20 μg/ml). The next day the cells were washed, fed with fresh media and maintained for 6 additional days, assaying for reverse transcriptase activity (RT) every 3 days. GHOST (3) cell lines express uniformly high levels of CD4 and low levels of endogenous CXCR4 (AIDS Research and Reference Reagent Program).

CD34+ cell isolation

CD34+ cells were mobilized with granulocyte-colony stimulating factor and/or granulocyte macrophage-colony stimulating factor from healthy volunteers after informed consent. Leukapheresis products were red cell depleted and CD34+ hematopoietic progenitor cells were isolated using the MACS CD34 progenitor cell-isolation kit, according to the manufacturer's instructions (Miltenyi Biotec). Briefly, 2×10^9 cells were magnetically labeled using hapten-conjugated CD34 monoclonal antibody (clone QBEND/10) and an anti-hapten antibody coupled to MACS MicroBeads. CD34-labeled cells were positively selected by passing them through MACS separation columns twice, to achieve 95% purity, and frozen in 90% fetal calf serum [(FCS) (Gibco, BRL)] and 10% DMSO (Sigma) until needed.

Confocal microscopy

Approximately 1×10^6 cells were centrifuged on to Superfrost Plus microscope slides (Fisher) using a StatSpin Cytofuge (StatSpin, Inc.). Cells were fixed in 100% acetone or 4% paraformaldehyde for 10 min and incubated in PBS/BSA/Tween 20 buffer [1× phosphate-buffered saline (PBS)/3% bovine serum albumin (BSA)/0.2% Tween 20] before the addition of antibodies. All antibody dilutions were made in PBS/BSA/Tween 20 buffer. Primary antibodies were incubated with cells for 1 h at room temperature and secondary antibodies were kept on cells for 30 min at room temperature. Excess antibody was removed by washing in PBS/BSA/Tween 20 buffer for 10 min by gentle agitation. The mouse monoclonal antibodies used were anti-human -CD41 (clone PM6/248, Chemicon International Inc.), -CD4 (clone QS4120, Calbiochem-Novabiochem Corp.), -CCR5 (clones 2D7 and 5C7, AIDS Research and Reference

Reagent Program), -CXCR4 (clone 44708.111, R&D Systems, Inc.), and -CCR3 (clone 7B11, AIDS Research and Reference Reagent Program). Isotype controls were performed using purified mouse IgG1 and IgG2a (Calbiochem). Pooled human sera from 10 HIV-1-infected patients were used as a source for antibodies against HIV-1 (the generous gift of Dr. Max Arens), which recognized HIV-1 p24 and gp120 as detected by immunoprecipitation of metabolically labeled virus and by Western blot analysis of patient sera (data not shown). Mouse monoclonal antibodies were labeled with anti-mouse IgG conjugated to phycoerythrin (PE) (Boehringer Mannheim) and HIV-1 antibodies were labeled using anti-human IgG conjugated to fluorescein isothiocyanate (FITC) (Calbiochem-Novabiochem Corp.). Slides were mounted using 1% n-propyl gallate solution (50 mM EDTA/50% glycerol/100 mM Tris pH 8.0) and antibody staining was detected by Laser Scanning Confocal microscopy (Zeiss Axiovert with Biorad confocal scanning imaging system).

Infection of megakaryocytes with recombinant HIV-1

CD34+ hematopoietic progenitor cells were cultured in Tpo media: StemPro-34 SFM serum-free medium (Gibco, BRL) supplemented with 50 μg/ml thrombopoietin (Tpo) (R&D Systems Inc.), 2 mM L-glutamine (Biowhittaker), and 100 μg/ml penicillin and streptomycin (Biowhittaker). At Day 7, 1×10^6 cells were infected with ~400 ng of HIV-1 $p24^{gag}$ overnight. The next day, the cells were washed extensively and cultured in Tpo media for 12–15 days. Every 3–4 days, one-third of the supernatant was replaced with fresh Tpo media and viral release was assayed by p24 ELISA (Coulter Corp.).

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REFERENCES

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., and Berger, E. A. (1996). CC CKR5: A RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**, 1955–1958.

Ballem, P. J., Belzberg, A., Devine, D. V., Lyster, D., Spruston, B., Chambers, H., Doubroff, P., and Mikulash, K. (1992). Kinetic studies of the mechanism of thrombocytopenia in patients with human immunodeficiency virus infection [see comments]. *N. Engl. J. Med.* **327,** 1779–1784.

Basch, R. S., Dolzhanskiy, A., Zhang, X. M., and Karpatkin, S. (1996). The development of human megakaryocytes. II. CD4 expression occurs during haemopoietic differentiation and is an early step in megakaryocyte maturation. *Br. J. Haematol.* **94,** 433–442.

Birkmann, J., Oez, S., Smetak, M., Kaiser, G., Kappauf, H., and Gallmeier, W. M. (1997). Effects of recombinant human thrombopoietin alone and in combination with erythropoietin and early-acting cytokines on human mobilized purified CD34+ progenitor cells cultured in serum-depleted medium. *Stem Cells* **15,** 18–32.

Boyar, A., and Beall, G. (1991). HIV-seropositive thrombocytopenia: the action of zidovudine. *Aids* **5,** 1351–1356.

Cairns, J. S., and D'Souza, M. P. (1998). Chemokines and HIV-1 second receptors: the therapeutic connection. *Nat. Med.* **4,** 563–568.

Chelucci, C., Casella, I., Federico, M., Testa, U., Macioce, G., Pelosi, E., Guerriero, R., Mariani, G., Giampaolo, A., Hassan, H. J., and Peschle, C. (1999). Lineage-specific expression of human immunodeficiency virus (HIV) receptor/coreceptors in differentiating hematopoietic precursors: correlation with susceptibility to T- and M-tropic HIV and chemokine-mediated HIV resistance. *Blood* **94,** 1590–1600.

Chelucci, C., Federico, M., Guerriero, R., Mattia, G., Casella, I., Pelosi, E., Testa, U., Mariani, G., Hassan, H. J., and Peschle, C. (1998). Productive human immunodeficiency virus-1 infection of purified megakaryocytic progenitors/precursors and maturing megakaryocytes. *Blood* **91,** 1225–1234.

Cheng-Mayer, C., Liu, R., Landau, N. R., and Stamatatos, L. (1997). Macrophage tropism of human immunodeficiency virus type 1 and utilization of the CC-CKR5 coreceptor. *J. Virol.* **71,** 1657–1661.

Cheng-Mayer, C., Seto, D., Tateno, M., and Levy, J. A. (1988). Biologic features of HIV-1 that correlate with virulence in the host. *Science* **240,** 80–82.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C., and Sodroski, J. (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85,** 1135–1148.

Choi, E. S., Hokom, M., Bartley, T., Li, Y. S., Ohashi, H., Kato, T., Nichol, J. L., Skrine, J., Knudten, A., Chen, J., *et al.* (1995a). Recombinant human megakaryocyte growth and development factor (rHuMGDF), a ligand for c-Mpl, produces functional human platelets in vitro. *Stem Cells* **13,** 317–322.

Choi, E. S., Nichol, J. L., Hokom, M. M., Hornkohl, A. C., and Hunt, P. (1995b). Platelets generated in vitro from proplatelet-displaying human megakaryocytes are functional. *Blood* **85,** 402–413.

Clapham, P. R., and Weiss, R. A. (1997). Immunodeficiency viruses. Spoilt for choice of co-receptors [news; comment]. *Nature* **388,** 230–231.

Cole, J. L., Marzec, U. M., Gunthel, C. J., Karpatkin, S., Worford, L., Sundell, I. B., Lennox, J. L., Nichol, J. L., and Harker, L. A. (1998). Ineffective platelet production in thrombocytopenic human immunodeficiency virus-infected patients. *Blood* **91,** 3239–3246.

Connor, R. I., Mohri, H., Cao, Y., and Ho, D. D. (1993). Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. *J Virol* **67,** 1772–1777.

Cramer, E. M., Norol, F., Guichard, J., Breton-Gorius, J., Vainchenker, W., Masse, J. M., and Debili, N. (1997). Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. *Blood* **89,** 2336–2346.

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312,** 763–767.

De Luca, A., Teofili, L., Antinori, A., Iovino, M. S., Mencarini, P., Visconti, E., Tamburrini, E., Leone, G., and Ortona, L. (1993). Haemopoietic CD34+ progenitor cells are not infected by HIV-1 in vivo but show impaired clonogenesis. *Br. J. Haematol.* **85,** 20–24.

Delwart, E. L., Mullins, J. I., Gupta, P., Learn, G. H., Jr., Holodniy, M., Katzenstein, D., Walker, B. D., and Singh, M. K. (1998). Human immunodeficiency virus type 1 populations in blood and semen. *J. Virol.* **72,** 617–623.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996). Identification of a major co-receptor for primary isolates of HIV-1 [see comments]. *Nature* **381,** 661–666.

Deng, H. K., Unutmaz, D., KewalRamani, V. N., and Littman, D. R. (1997). Expression cloning of new receptors used by simian and human immunodeficiency viruses [see comments]. *Nature* **388,** 296–300.

Dittmar, M. T., Simmons, G., Donaldson, Y., Simmonds, P., Clapham, P. R., Schulz, T. F., and Weiss, R. A. (1997). Biological characterization of human immunodeficiency virus type 1 clones derived from different organs of an AIDS patient by long-range PCR. *J. Virol.* **71,** 5140–5147.

Donaldson, Y. K., Bell, J. E., Ironside, J. W., Brettle, R. P., Robertson, J. R., Busuttil, A., and Simmonds, P. (1994). Redistribution of HIV outside the lymphoid system with onset of AIDS. *Lancet* **343,** 383–385.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Doms, R. W. (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85,** 1149–1158.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P., and Paxton, W. A. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5 [see comments]. *Nature* **381,** 667–673.

Dumitrescu, O., Kalish, M. L., Kliks, S. C., Bandea, C. I., and Levy, J. A. (1994). Characterization of human immunodeficiency virus type 1 isolates from children in Romania: Identification of a new envelope subtype. *J. Infect. Dis.* **169,** 281–288.

Fauci, A. S. (1996). Host factors and the pathogenesis of HIV-induced disease. *Nature* **384,** 529–534.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996). HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor [see comments]. *Science* **272,** 872–877.

Frade, J. M. R., Llorente, M., Mellado, M., Alcami, J., Gutierrez-Ramos, J. C., Zaballos, A., Real, G., and Martinez, A. C. (1997). The amino-terminal domain of the CCR2 chemokine receptor acts as coreceptor for HIV-1 infection. *J. Clin. Invest.* **100,** 497–502.

Gewirtz, A. M., Boghosian-Sell, L., Catani, L., Ratajczak, M. Z., Shen, Y. M., and Schreiber, A. D. (1992). Expression of Fc gamma RII and CD4 receptors by normal human megakaryocytes. *Exp. Hematol.* **20,** 512–516.

Harbol, A. W., Liesveld, J. L., Simpson-Haidaris, P. J., and Abboud, C. N. (1994). Mechanisms of cytopenia in human immunodeficiency virus infection. *Blood Rev.* **8,** 241–251.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., Busciglio, J., Yang, X., Hofmann, W., Newman, W., Mackay, C. R., Sodroski, J., and Gabuzda, D. (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385,** 645–649.

Horuk, R., Hesselgesser, J., Zhou, Y., Faulds, D., Halks-Miller, M., Harvey, S., Taub, D., Samson, M., Parmentier, M., Rucker, J., Doranz, B. J., and Doms, R. W. (1998). The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV-1 strains. *J. Biol. Chem.* **273,** 386–391.

Huang, Y., and Carmichael, G. C. (1996). Role of polyadenylation in nucleocytoplasmic transport of mRNA. *Mol. Cell. Biol.* **16,** 1534–1542.

Hung, C.-S., Vander Heyden, N., and Ratner, L. (1999). Relationship between productive HIV-1 infection of macrophages and CCR5 utilization. *Virol.* **264,** 278–288.

Jenkins, M., Hanley, M. B., Moreno, M. B., Wieder, E., and McCune, J. M. (1998). Human immunodeficiency virus-1 infection interrupts thymopoiesis and multilineage hematopoiesis in vivo. *Blood* **91,** 2672–2678.

Jinno, A., Shimizu, N., Soda, Y., Haraguchi, Y., Kitamura, T., and Hoshino, H. (1998). Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. *Biochem. Biophys. Res. Commun.* **243,** 497–502.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. C., and Montagnier, L. (1984). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* **312,** 767–768.

Koka, P. S., Fraser, J. K., Bryson, Y., Bristol, G. C., Aldrovandi, G. M., Daar, E. S., and Zack, J. A. (1998). Human immunodeficiency virus inhibits multilineage hematopoiesis in vivo. *J. Virol.* **72,** 5121–5127.

Kouri, Y. H., Borkowsky, W., Nardi, M., Karpatkin, S., and Basch, R. S. (1993). Human megakaryocytes have a CD4 molecule capable of binding human immunodeficiency virus-1. *Blood* **81,** 2664–2670.

Kowalska, M. A., Ratajczak, J., Hoxie, J., Brass, L. F., Gewirtz, A., Poncz, M., and Ratajczak, M. Z. (1999). Megakaryocyte precursors, megakaryocytes and platelets express the HIV co-receptor CXCR4 on their surface: Determination of response to stromal-derived factor-1 by megakaryocytes and platelets. *Br. J. Haematol.* **104,** 220–229.

Lee, B., Doranz, B. J., Ratajczak, M. Z., and Doms, R. W. (1998). An intricate Web: Chemokine receptors, HIV-1 and hematopoiesis. *Stem Cells* **16,** 79–88.

Lee, B., Ratajczak, J., Doms, R. W., Gewirtz, A. M., and Ratajczak, M. Z. (1999). Coreceptor/chemokine receptor expression on human hematopoietic cells: Biological implications for human immunodeficiency virus-type 1 infection. *Blood* **93,** 1145–1156.

Liao, F., Alkhatib, G., Peden, K. W., Sharma, G., Berger, E. A., and Farber, J. M. (1997). STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* **185,** 2015–2023.

Loetscher, M., Amara, A., Oberlin, E., Brass, N., Legler, D., Loetscher, P., D'Apuzzo, M., Meese, E., Rousset, D., Virelizier, J. L., Baggiolini, M., Arenzana-Seisdedos, F., and Moser, B. (1997). TYMSTR, a putative chemokine receptor selectively expressed in activated T cells, exhibits HIV-1 coreceptor function. *Curr. Biol.* **7,** 652–660.

Louache, F., Bettaieb, A., Henri, A., Oksenhendler, E., Farcet, J. P., Bierling, P., Seligmann, M., and Vainchenker, W. (1991). Infection of megakaryocytes by human immunodeficiency virus in seropositive patients with immune thrombocytopenic purpura. *Blood* **78,** 1697–1705.

Louache, F., Henri, A., Bettaieb, A., Oksenhendler, E., Raguin, G., Tulliez, M., and Vainchenker, W. (1992). Role of human immunodeficiency virus replication in defective in vitro growth of hematopoietic progenitors. *Blood* **80,** 2991–2999.

Maddon, P. J., Dalgleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A., and Axel, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47,** 333–348.

McKnight, A., Wilkinson, D., Simmons, G., Talbot, S., Picard, L., Ahuja, M., Marsh, M., Hoxie, J. A., and Clapham, P. R. (1997). Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. *J. Virol.* **71,** 1692–1696.

Michael, N. L., Nelson, J. A., KewalRamani, V. N., Chang, G., O'Brien, S. J., Mascola, J. R., Volsky, B., Louder, M., White, G. C., 2nd, Littman, D. R., Swanstrom, R., and O'Brien, T. R. (1998). Exclusive and persistent use of the entry coreceptor CXCR4 by human immunodeficiency virus type 1 from a subject homozygous for CCR5 delta32. *J. Virol.* **72,** 6040–6047.

Molina, J. M., Scadden, D. T., Sakaguchi, M., Fuller, B., Woon, A., and Groopman, J. E. (1990). Lack of evidence for infection of or effect on growth of hematopoietic progenitor cells after in vivo or in vitro exposure to human immunodeficiency virus. *Blood* **76,** 2476–2482.

Monte, D., Groux, H., Raharinivo, B., Plouvier, B., Dewulf, J., Clavel, T., Grangette, C., Torpier, G., Auriault, C., Capron, A., *et al.* (1992). Productive human immunodeficiency virus-1 infection of megakaryocytic cells is enhanced by tumor necrosis factor-alpha. *Blood* **79,** 2670–2679.

Moses, A., Nelson, J., and Bagby, G. C., Jr. (1998). The influence of human immunodeficiency virus-1 on hematopoiesis. *Blood* **91,** 1479–1495.

Mummidi, S., Ahuja, S. S., Gonzalez, E., Anderson, S. A., Santiago, E. N., Stephan, K. T., Craig, F. E., O'Connell, P., Tryon, V., Clark, R. A., Dolan, M. J., and Ahuja, S. K. (1998). Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nat. Med.* **4,** 786–793.

Oksenhendler, E., Bierling, P., Ferchal, F., Clauvel, J. P., and Seligmann, M. (1989). Zidovudine for thrombocytopenic purpura related to hu-

man immunodeficiency virus (HIV) infection. *Ann. Intern. Med.* **110,** 365–368.

Poss, M., Martin, H. L., Kreiss, J. K., Granville, L., Chohan, B., Nyange, P., Mandaliya, K., and Overbaugh, J. (1995). Diversity in virus populations from genital secretions and peripheral blood from women recently infected with human immunodeficiency virus type 1. *J. Virol.* **69,** 8118–8122.

Ratner, L. (1989). Human immunodeficiency virus-associated autoimmune thrombocytopenic purpura: A review. *Am. J. Med.* **86,** 194–198.

Riviere, C., Subra, F., Cohen-Solal, K., Cordette-Lagarde, V., Letestu, R., Auclair, C., Vainchenker, W., and Louache, F. (1999). Phenotypic and functional evidence for the expression of CXCR4 receptor during megakaryocytopoiesis. *Blood* **93,** 1511–1523.

Rucker, J., Edinger, A. L., Sharron, M., Samson, M., Lee, B., Berson, J. F., Yi, Y., Margulies, B., Collman, R. G., Doranz, B. J., Parmentier, M., and Doms, R. W. (1997). Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. *J. Virol.* **71,** 8999–9007.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C. M., Saragosti, S., Lapoumeroulie, C., Cognaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collman, R. G., Doms, R. W., Vassart, G., and Parmentier, M. (1996). Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene [see comments]. *Nature* **382,** 722–725.

Schipper, L. F., Brand, A., Reniers, N. C., Melief, C. J., Willemze, R., and Fibbe, W. E. (1998). Effects of thrombopoietin on the proliferation and differentiation of primitive and mature haemopoietic progenitor cells in cord blood. *Br. J. Haematol.* **101,** 425–435.

Shieh, J. T., Albright, A. V., Sharron, M., Gartner, S., Strizki, J., Doms, R. W., and Gonzalez-Scarano, F. (1998). Chemokine receptor utilization by human immunodeficiency virus type 1 isolates that replicate in microglia. *J. Virol.* **72,** 4243–4249.

Simmons, G., Wilkinson, D., Reeves, J. D., Dittmar, M. T., Beddows, S., Weber, J., Carnegie, G., Desselberger, U., Gray, P. W., Weiss, R. A., and Clapham, P. R. (1996). Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J. Virol.* **70,** 8355–8360.

Smith, M. W., Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Lomb, D. A., Goedert, J. J., O'Brien, T. R., Jacobson, L. P., Kaslow, R., Buchbinder, S., Vittinghoff, E., Vlahov, D., Hoots, K., Hilgartner, M. W., and O'Brien, S. J. (1997). Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* **277,** 959–965.

Smyth, R. J., Yi, Y., Singh, A., and Collman, R. G. (1998). Determinants of entry cofactor utilization and tropism in a dualtropic human immunodeficiency virus type 1 primary isolate. *J. Virol.* **72,** 4478–4484.

Speck, R. F., Wehrly, K., Platt, E. J., Atchison, R. E., Charo, I. F., Kabat, D., Chesebro, B., and Goldsmith, M. A. (1997). Selective employment of chemokine receptors as human immunodeficiency virus type 1 coreceptors determined by individual amino acids within the envelope V3 loop. *J. Virol.* **71,** 7136–7139.

Stewart, G. J., Ashton, L. J., Biti, R. A., Ffrench, R. A., Bennetts, B. H., Newcombe, N. R., Benson, E. M., Carr, A., Cooper, D. A., and Kaldor, J. M. (1997). Increased frequency of CCR-5 delta 32 heterozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-Term Non-Progressor Study Group. *AIDS* **11,** 1833–1838.

van't Wout, A. B., Ran, L. J., Kuiken, C. L., Kootstra, N. A., Pals, S. T., and Schuitemaker, H. (1998). Analysis of the temporal relationship between human immunodeficiency virus type 1 quasispecies in sequential blood samples and various organs obtained at autopsy. *J. Virol.* **72**, 488–496.

Voulgaropoulou, F., Tan, B., Soares, M., Hahn, B., and Ratner, L. (1999). Distinct human immunodeficiency virus strains in the bone marrow are associated with the development of thrombocytopenia. *J. Virol.* **73,** 3497–3504.

Walsh, C. M., Nardi, M. A., and Karpatkin, S. (1984). On the mechanism of thrombocytopenic purpura in sexually active homosexual men. *N. Engl. J. Med.* **311,** 635–639.

Wang, J. F., Liu, Z. Y., and Groopman, J. E. (1998). The alpha-chemokine receptor CXCR4 is expressed on the megakaryocytic lineage from progenitor to platelets and modulates migration and adhesion. *Blood* **92,** 756–764.

Xiao, L., Owen, S. M., Goldman, I., Lal, A. A., deJong, J. J., Goudsmit, J., and Lal, R. B. (1998). CCR5 coreceptor usage of non-syncytium-inducing primary HIV-1 is independent of phylogenetically distinct global HIV-1 isolates: Delineation of consensus motif in the V3 domain that predicts CCR-5 usage. *Virology* **240,** 83–92.

Zauli, G., Catani, L., Gibellini, D., Re, M. C., Milani, D., Borgatti, P., Bassini, A., La Placa, M., and Capitani, S. (1995). The CD4 receptor plays essential but distinct roles in HIV-1 infection and induction of apoptosis in primary bone marrow GPIIb/IIIa+ megakaryocytes and the HEL cell line. *Br. J. Haematol.* **91,** 290–298.

Zauli, G., Catani, L., Gibellini, D., Re, M. C., Vianelli, N., Colangeli, V., Celeghini, C., Capitani, S., and La Placa, M. (1996). Impaired survival of bone marrow GPIIb/IIa+ megakaryocytic cells as an additional pathogenetic mechanism of HIV-1-related thrombocytopenia. *Br. J. Haematol.* **92,** 711–717.

Zauli, G., and Davis, B. R. (1993). Role of HIV infection in the hematologic manifestations of HIV seropositive subjects. *Crit. Rev. Oncol. Hematol.* **15,** 271–283.

Zauli, G., Re, M. C., Davis, B., Sen, L., Visani, G., Gugliotta, L., Furlini, G., and La Placa, M. (1992a). Impaired in vitro growth of purified (CD34+) hematopoietic progenitors in human immunodeficiency virus-1 seropositive thrombocytopenic individuals. *Blood* **79,** 2680–2687.

Zauli, G., Re, M. C., Furlini, G., Giovannini, M., and La Placa, M. (1992b). Human immunodeficiency virus type 1 envelope glycoprotein gp120-mediated killing of human haematopoietic progenitors (CD34+ cells). *J. Gen. Virol.* **73,** 417–421.

Zauli, G., Re, M. C., Gugliotta, L., Visani, G., Vianelli, N., Furlini, G., and La Placa, M. (1991). Lack of compensatory megakaryocytopoiesis in HIV-1-seropositive thrombocytopenic individuals compared with immune thrombocytopenic purpura patients. *AIDS* **5,** 1345–1350.

Zauli, G., Re, M. C., Visani, G., Furlini, G., Mazza, P., Vignoli, M., and La Placa, M. (1992c). Evidence for a human immunodeficiency virus type 1-mediated suppression of uninfected hematopoietic (CD34+) cells in AIDS patients. *J. Infect. Dis.* **166,** 710–716.

Zauli, G., Vitale, M., Falcieri, E., Gibellini, D., Bassini, A., Celeghini, C., Columbaro, M., and Capitani, S. (1997). In vitro senescence and apoptotic cell death of human megakaryocytes. *Blood* **90,** 2234–2243.

Zhang, L., He, T., Talal, A., Wang, G., Frankel, S. S., and Ho, D. D. (1998). In vivo distribution of the human immunodeficiency virus/simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. *J. Virol.* **72,** 5035–5045.

Zhang, L., Huang, Y., He, T., Cao, Y., and Ho, D. D. (1996). HIV-1 subtype and second-receptor use [letter]. *Nature* **383,** 768.

Zhang, Y. J., and Moore, J. P. (1999). Will multiple coreceptors need to be targeted by inhibitors of human immunodeficiency virus type 1 entry? *J. Virol.* **73**, 3443–3448.

Zucker-Franklin, D., and Cao, Y. Z. (1989). Megakaryocytes of human immunodeficiency virus-infected individuals express viral RNA. *Proc. Natl. Acad. Sci. USA* **86,** 5595–5599.

Zucker-Franklin, D., and Kaushansky, K. (1996). Effect of thrombopoietin on the development of megakaryocytes and platelets: An ultrastructural analysis. *Blood* **88,** 1632–1638.

Zucker-Franklin, D., Termin, C. S., and Cooper, M. C. (1989). Structural changes in the megakaryocytes of patients infected with the human immune deficiency virus (HIV-1). *Am. J. Pathol.* **134,** 1295–1303.